Preformulation Studies and Prototype Formulation Development of Nevirapine Loaded Chitosan Based Nanoparticle for Vaginal Drug Delivery System

Keywords: Preformulation studies, Nevirapine loaded chitosan nanoparticle, In-vitro drug release

ABSTRACT

Nanoparticles (NPs) have the potential to provide effective and safe delivery of antiretroviral drugs in the context of prophylactic anti-HIV vaginal infection. The aim of this proposed research study is to carried out the pre formulation studies and prototype development of nevirapine (NVP) loaded chitosan nanoparticle for the vaginal delivery system to prevent HIV infection and also limit the occurrence of severity of side effects associated with systemic exposure to anti-HIV product. In this study, NVP loaded chitosan nanoparticle was prepared by an emulsion-diffusion evaporation method and characterized for drug-excipients compatibility testing by Fourier transform (FT-IR) spectroscopy. The results of FT-IR study confirm the absence of interactions. All the physicochemical properties of the drug were found within the official compendia. The solubility studies revealed that the solubility of the drug is pH dependent and is more soluble in the acidic pH. The results of solution phase stability studies proved that the drug is more stable at acidic pH. The maximum percentage yield and encapsulation efficiency with F3 formulation was found to be 85.03±0.02% and 43.4±1.6% respectively. The average size of prepared nanoparticles varied from 489±6.19 nm to 651.8±6.33nm with a polydispersity index (PI) in the range of 0.420.011± to 0.845±0.012. In-vitro drug release of F3 formulation was found to be 97.82±1.2% in phosphate buffer pH 4.5. In conclusion, results of pre-formulation studies showed the feasibility of Nevirapine to develop a vaginal nanoparticle dosage form which expected to reduce the systemic toxicity of the drug.
INTRODUCTION

HIV affects over 33 million people worldwide. Close to 3 million new HIV infections and 2 million AIDS-related deaths occur each year. Leading the world in HIV incidence rates, sub-Saharan Africa accounted for 71% of new infections in 2008. Sexual transmission remains a leading cause of HIV infection and females, especially African women and girls, are disproportionately affected by this disease accounting for approximately 60% infections in sub-Saharan Africa. This feminization of the HIV/AIDS pandemic is fueled by the fact that females are at a greater infection risk than men, with young females at the most risk. Physiological susceptibility and social, legal and economic disadvantages make women more likely to become infected [1, 2]. Prevention of this life-threatening disease is critical in order to change the course of this pandemic. Abstinence, reduction of the number of sexual partners and concurrent sexual relationships, and correct, consistent condom use are highly effective against HIV acquisition but has proven to be insufficient to combat this incurable disease [3, 4, 5]. Development of safe, effective and acceptable female-controlled prevention methods, specifically those applied vaginally will play a major role in reducing the incidence of HIV-1 transmission.

In the nearly 100 years since the early descriptions of drug absorption occurring after topical vaginal administration, research on vaginal drug delivery has recently intensified. Vaginal drug administration has many advantages over the conventional oral administration, such as avoidance of the harsh gastrointestinal (GI) environment and the hepatic first-pass effect. Absorption from the GI tract can be affected by fed state, drug–drug interactions, microbiota, and GI disturbances. Further, the fraction of drug that is absorbed from the GI tract can then be metabolized and eliminated by first passage through the liver. Thus, vaginal drug administration allows for smaller drug doses and the potential for reduced side effects. Additionally, for drugs that act locally in the female reproductive tract, topical vaginal application results in much higher drug concentrations and improved efficacy [6].

Aim and objectives:

The aim of this proposed research study is taken as to develop a novel vaginal delivery system of an antiretroviral drug to prevent HIV infection and also limit the occurrence of the severity of side effects associated with systemic exposure to anti-HIV product.
MATERIALS AND METHODS

Chemicals and glassware:

NVP was obtained from Novartis (Basel, Switzerland) and Chitosan was purchased from Sigma–Aldrich (Denmark). All chemicals and solvents used in this study were of analytical grade. HPLC-grade acetonitrile, methanol, potassium phthalate and ammonium acetate were purchased from Sigma (Denmark). Glacial acetic acid (purity 99.8%) was obtained from Merck (Darmstadt, Germany). Phosphate buffer solution (pH4.5) was prepared by mixing appropriate amounts of ammonium acetate and acetic acid.

Preformulation testing:

Methodology

Organoleptic Properties

The organoleptic properties of the received API sample were evaluated for colour, odour and taste by visual observation.

Melting point determination of Nevirapine

Melting point of nevirapine was determined by melting point apparatus (Macro scientific works10A/UA, Janwahar Nagar, Delhi-11007) using capillary tube method and compared with the melting point of nevirapine reported in the literature.

Differential scanning calorimetric study

A differential scanning calorimeter (JADE DSC, PerkinElmer, USA) was used for thermal analysis of nevirapine and nevirapine-excipients mixtures. Excipients expected to be used in the development of NVP loaded nano gel at an appropriate ratio were selected for the study\(^7,\)\(^8\). Individual samples (Nevirapine and excipients) as well as physical mixtures of nevirapine and selected excipients (all passed through 60-mesh sieve) were weighed directly in the pierced DSC aluminium pan (Table1) and scanned in the temperature range of 20-300\(^0\) C under an atmosphere of dry nitrogen. The heating rate of 10\(^0\) C/min was used and thermograms obtained were observed for any interactions.
Table 1: Peak temperature and enthalpy values of nevirapine in various drug-excipient mixtures

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ratio (drug-excipient)</th>
<th>$T_{\text{onset}}$ (°C)</th>
<th>$T_{\text{peak}}$ (°C)</th>
<th>$\Delta H_{\text{fcorr}}$ (Jg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NVP</td>
<td>-----</td>
<td>245.56</td>
<td>248.36</td>
<td>128.22</td>
</tr>
<tr>
<td>NVP + Chitosan</td>
<td>1:2</td>
<td>245.15</td>
<td>246.87</td>
<td>62.42</td>
</tr>
<tr>
<td>NVP + Pluronic F127</td>
<td>1:3</td>
<td>62.77</td>
<td>59.67</td>
<td>195.01</td>
</tr>
</tbody>
</table>

$\Delta H_{\text{fcorr}} = \Delta H_{\text{obs}} / \text{drug conc. in sample (g/100g)}$

**FT-IR spectroscopy**

FT-IR spectra of the drug and a blend of drug and selected excipient were recorded on a Bruker Spectrophotometer (Model-220, Germany) in the range of 4000-400 cm$^{-1}$ using Potassium bromide Discs.

**Isothermal stress testing**

In the IST studies drug and different excipients (Table 2) were weighed directly in 4ml glass vials ($n = 2$) and mixed on a vortex mixer for 2 min. In each of the vials, water (10% v/w) was added and the drug-excipients blend was further mixed with a glass capillary (both the ends of which were heat sealed) [9]. To prevent any loss of material, capillary was broken and left inside the vial. Each vial was sealed using a Teflon-lined screw cap and stored at 50 °C in a hot air oven. These samples were periodically examined for any unusual color change. After 3 weeks of storage at the above conditions, samples were quantitatively analyzed using a UV-Visible spectrophotometer. Drug-excipients blends without added water stored in refrigerator served as controls.

Table 2: UV analysis of the samples under IST conditions after 3 weeks of storage

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ratio (drug-excipient)</th>
<th>Drug remaining</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control value$^6$</td>
<td>Stressed samples$^6$</td>
<td></td>
</tr>
<tr>
<td>NVP</td>
<td>..</td>
<td>100.08±0.8</td>
<td>98.02±0.06</td>
<td></td>
</tr>
<tr>
<td>NVP + Chitosan</td>
<td>1:2</td>
<td>101.03±0.21</td>
<td>97.62±0.11</td>
<td></td>
</tr>
<tr>
<td>NVP + Carbopol 974P</td>
<td>1:3</td>
<td>99.02±0.11</td>
<td>98.59±0.13</td>
<td></td>
</tr>
<tr>
<td>NVP + PVA</td>
<td>1:1</td>
<td>102.03±0.18</td>
<td>99.69±0.23</td>
<td></td>
</tr>
<tr>
<td>NVP + Pluronic F127</td>
<td>1:1</td>
<td>101.50±0.13</td>
<td>97.21±0.31</td>
<td></td>
</tr>
</tbody>
</table>

Citation: Sheikh Sofiur Rahman et al. Ijppr.Human, 2016; Vol. 7 (1): 584-596.
For sample preparation, 2ml of methanol was added into each vial. The mixture was vortexed for 3 min and transferred to 100ml volumetric flask. Vials were rinsed twice with methanol and the volume made up. The samples were centrifuged and the supernatant filtered through 0.45µm nylon membrane filters. After appropriate dilutions, samples were analyzed in UV-Visible spectrophotometer at 254 nm wavelength and drug content was determined from the calibration curve prepared within the expected range.

**Prototype formulation development of drug loaded nanoparticles**

Prototype nanoparticle was developed by using various excipients selected from compatibility studies \[^{10}\]. NVP loaded chitosan nanoparticles were prepared by the emulsion–diffusion–evaporation method as per the composition is shown in table 3. In brief, 200 mg of nevirapine and 200-600 mg of chitosan (1:1 to 1:3, drug to polymer ratio) were dissolved in 25 ml of an acetone-acetic acid mixture (1:2 ratios) at room temperature for 2 h. The organic phase was then added to 50 ml of an aqueous phase containing pluronic F127 (6% w/v) as a stabilizer. The resulting primary o/w emulsion was stirred at 1000rpm for 1hr and subsequently homogenized at 24,000 rpm for 5 min using a high-speed homogenizer (IKA T25 digital Ultra Turrax, Germany). To this emulsion, water was added with constant stirring to facilitate diffusion and finally evaporate the organic solvent. This resulted in polymer precipitation and formation of nanoparticles. Free drug and surfactant were separated by centrifuging (REMI cooling centrifuge, Vasai) the drug-loaded nanoparticles at 10,000×g for 20 min.
Table 3: Composition of prototype drug loaded chitosan nanoparticles.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Nevirapine (mg)</th>
<th>Chitosan (mg)</th>
<th>Pluronic F127 (mg)</th>
<th>Acetone-acetic acid mixture (1:2 ratio) ml</th>
<th>Distilled water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>200</td>
<td>200</td>
<td>1</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>F2</td>
<td>200</td>
<td>400</td>
<td>1</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>F3</td>
<td>200</td>
<td>600</td>
<td>1</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>F4</td>
<td>200</td>
<td>200</td>
<td>2</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>F5</td>
<td>200</td>
<td>400</td>
<td>2</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>F6</td>
<td>200</td>
<td>600</td>
<td>2</td>
<td>25</td>
<td>50</td>
</tr>
</tbody>
</table>

Characterization of nanoparticle:

Particle size determination

Hydrodynamic diameter and polydispersity index of nevirapine nanoparticles were determined by using zetasizer Nano ZS 90 dynamic light scattering equipment \[^{11, 12}\] (Malvern Instruments Ltd. UK). Nanoparticles (~1 mg/mL) were dispersed in distilled water using sonication prior to particle size. Mean hydrodynamic diameters and polydispersity index were calculated based on size distribution by weight, assuming a lognormal distribution and the results were expressed as mean ± S.D. of five runs.

Encapsulation efficiency

Loading of Nevirapine in chitosan nanoparticles were determined by extracting 10 ml nanoparticles with 1 ml acetone for 6 hrs. From this solution, 0.2 ml was diluted with phosphate buffer pH 4.5 and analyzed by UV spectrophotometer (Shimadzu UV-1800, Japan) at 254 nm against appropriate blank \[^{13,14}\]. Drug loading and encapsulation efficiency were calculated by using equation Eq. (1) and Eq. (2) respectively:

\[
\% \text{ Drug Loading} = \frac{\text{Amount of drug in nanoparticle (mg)}}{\text{Amount of nanoparticle}} \quad \text{Eq. (1)}
\]

\[
\% \text{ Entrapment Efficiency} = \frac{\text{Amount of drug in nanoparticles (mg)}}{\text{Initial mount of drug (mg)}} \quad \text{Eq. (2)}
\]
In vitro release

The in-vitro drug release studies of Nevirapine loaded chitosan nanoparticles was determined in phosphate buffer pH 4.5 at 37 °C[15, 16]. Nanoparticle suspension (1mg/ml, 0.5 ml) was placed in a dialysis tube (cellulose membrane, Sigma Chemical Company, USA) and immersed in 10 ml of the release buffer in a 15-ml centrifuge tube and shaken in an incubator shaker set at 100 rpm. At predetermined time intervals, 1 ml of the buffer solution was removed from the tube and analyzed for drug content by UV spectrophotometer (Shimadzu UV-1800, Japan) at 254 nm against appropriate blank[17].

RESULT AND DISCUSSION

The physicochemical properties of the drug Nevirapine are carried out and all the results were found satisfactory with respect to official compendia.

Differential scanning calorimetric study

DSC thermograms of drug and drug–excipients mixtures are shown in Figures 1-3. The thermal behaviour of pure drug, respective excipients, and the combination of drug and excipients was compared in the DSC thermograms. peak transition temperature (T_peak) and heat of fusion or enthalpy (ΔHf) of nevirapine in various excipients mixtures are summarized. The DSC trace of nevirapine showed a first endothermic event between 240 and 250°C with a melting point temperature of (Tonset=245.58°C). This endothermic peak was also retained in the entire drug–excipients with a little shifting of the peaks which may be due to the presence of moisture or impurity of the excipient.

The DSC thermogram of nevirapine-chitosan mixture showed an endothermic peak of nevirapine at 246.87 indicating that nevirapine is compatible with chitosan. The comparative curve of nevirapine, nevirapine–chitosan mixture is shown.

The DSC thermogram of Pluronic F127 showed endothermic peaks at 52.77 and 59.87 (melting point). Melting endothermic peak of Nevirapine lay at 242.48 in the nevirapine-pluronic F127 mixture suggesting that there was no interaction between the nevirapine and pluronic F127.
Figure 1: DSC thermogram of Nevirapine

Figure 2: DSC thermogram of NVP-Chitosan

Figure 3: DSC thermogram of NVP-Pluronic F127
In the majority of cases, the melting endotherm of the drug ($T_{\text{onset}}$ and $T_{\text{peak}}$) was also well preserved with slight broadening or shifting towards the lower temperature range. It has been reported that the quantity of material used, especially in drug-excipient mixtures, affects the peak shape and enthalpy. Thus these minor changes in the melting endotherm of drug could be due to the mixing of drug and excipient, which lower the purity of each component in the mixture and may not necessarily indicate potential incompatibility. The results of DSC studies were further correlated with FT-IR and IST studies.

**Isothermal stress testing**

The results of isothermal stress testing (IST) studies are showed in table 2. The content of drug in all the nevirapine-excipients mixture were found in the range of 99.02 ±0.11to 102.03 ± 0.18in controlled condition (Refrigerator, 2-8 °C) and in a stressed condition (stored at 50°C) was 97.21±0.31 to 99.69± 0.23 %. The results showed 3-5% variations in stressed condition with respect to controlled condition and the difference in drug content in the mentioned conditions was found statistically significant p= 0.0032 (p< 0.05), indicating the decomposition of nevirapine at the stressed condition. This result was in agreement with ahmed and nath [7].

Results of Isothermal Stress Testing studies showed the drug content is within the limit which revealed that all the excipients are compatible with each other.

**FT-IR spectroscopic study of drug-excipients mixture**

FT-IR spectrum of nevirapine is shown in Figure 4 and the following characteristics band were observed 1643.70 cm$^{-1}$ for (C=O stretching, aromatic/cyclic amide); 1464.56 cm$^{-1}$ (C=C stretching, aromatic), 1410.03 cm$^{-1}$ (skeletal vibration stretching), 1288.24cm$^{-1}$ (C-N, stretching), 1209.63cm$^{-1}$ (C-H in plane bending), 2950 cm$^{-1}$(N-H peak). The above characteristics band for Nevirapine is also found in various Nevirapine-excipients mixture. The comparative FT-IR spectra of Nevirapine and various drug excipients mixture were shown in figures 5 to 6. Indicating the absence of interaction.
Figure 4: FTIR Spectra of Nevirapine

Figure 5: FTIR spectra of Chitosan (A), Chitosan+ Nevirapine (B), Nevirapine(C)

Figure 6: FTIR spectra of Pluronic F127 (A), Pluronic F127+ NVP (B), and Nevirapine(C)
Table 4: Physico-chemical Characterization of NVP loaded chitosan nanoparticles

<table>
<thead>
<tr>
<th>F.No</th>
<th>D:Pol ratio</th>
<th>Size (nm)</th>
<th>Yield (%)</th>
<th>EE (%)</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1:1</td>
<td>489.0±6.19</td>
<td>67±0.08</td>
<td>15.4±1.2</td>
<td>0.618±0.002</td>
</tr>
<tr>
<td>F2</td>
<td>1:2</td>
<td>491.6±7.12</td>
<td>69±0.07</td>
<td>16.1±1.3</td>
<td>0.845±0.012</td>
</tr>
<tr>
<td>F3</td>
<td>1:3</td>
<td>522.4±8.23</td>
<td>73±0.05</td>
<td>23.09±3.9</td>
<td>0.425±0.011</td>
</tr>
<tr>
<td>F4</td>
<td>1:1</td>
<td>539.9±9.21</td>
<td>79.1±0.05</td>
<td>37.9±1.4</td>
<td>0.735±0.013</td>
</tr>
<tr>
<td>F5</td>
<td>1:2</td>
<td>588.9±8.24</td>
<td>81.01±0.03</td>
<td>38.1±1.4</td>
<td>0.768±0.007</td>
</tr>
<tr>
<td>F6</td>
<td>1:3</td>
<td>651.8±6.33</td>
<td>85.03±0.02</td>
<td>43.4±1.6</td>
<td>0.658±0.009</td>
</tr>
</tbody>
</table>


The physic-chemical characteristics of NVP loaded chitosan nanoparticles were summarized shown in Table 4. The result showed the high yield of nanoparticle i.e. 67±0.08 to 85.03±0.02%. The average loading of nanoparticles were about 5±1.1%, 10±%0.94, 14±0.97%, 15±1.0%, 17±0.89% and encapsulation efficiency of 15.4±2.4%, 16.1±4.3%, 23.09±3.5%, 37.9±4.8%, 38.1±4.1%, 43.4±4.8% in formulation F1, F2, F3, F4, F5, F6 respectively. The average loading and encapsulation efficiency were found to be increased with an increase in polymer as well as stabilizer concentration used in the formulations. A maximum of 17±0.89% drug loading and 43.4±4.8% entrapment efficiency was observed at 1:3, drug to polymer ratio in formulation F6, the change in drug loading may be due to the poor aqueous solubility of the drug and high binding capacity of drug on polymer surface in organic solvent used in the nanoparticle formation.

The result showed the average size of prepared nanoparticles varied from 489±6.19 nm to 651.8± nm with a polydispersity index (PI) in the range of 0.425±0.011 to 0.845±0.012 as shown in Table 4. The size of nanoparticle was increased with increase in polymer concentration. Nevertheless, the PI was found to be always lower than 1, which indicates homogeneous nanoparticle formulation. The addition of Pluronic F127 in the formulation aids to reduce aggregation of nanoparticles may result in the stability of the formulation.

**In-Vitro drug release studies**

*In vitro* release profiles of NVP loaded chitosan nanoparticles in phosphate buffer pH 4.5 is shown in Figure 7. Controlled release of drug was observed from the formulations for the
duration of 6 hrs. It was found that as the concentration of polymer increased in the formulations drug release was controlled for a longer period, which may be due to the hydration ability of chitosan, which on coming in contact with dissolution media leads to the formation of gelatinous mass and act as retardant material for the drug to diffuse out. Thus a prolonged release of the drug is attained and at the end of 6 hrs, the cumulative % drug release from formulations F1, F2, F3 were 83.13, 87.74, and 97.82 respectively. Thus F3 formulation contained a higher percentage of polymer (1:3, Drug: polymer ratio) was able to control the release of drug for a long period of time (more than 6 hrs) compared to other formulations in phosphate buffer pH.

Figure 7: In-Vitro drug release profile for different formulation in Phosphate buffer, pH 4.5

CONCLUSION

In this study, all the physicochemical properties of the drug Nevirapine is carried out and all the results were found satisfactory with respect to Indian Pharmacopoeia. The melting point of the drug was found 247-249 °C, which matched the melting point as reported in official pharmacopoeia (B.P). Based on the results of DSC, IST and FT-IR studies it revealed that all the excipients used in the formulations are compatible with nevirapine. The average size of prepared nanoparticles varied from 489±6.19 nm to 651.8±6.33 nm with a polydispersity index (PI) in the range of 0.425±0.011 to 0.845±0.012. From the in-vitro drug release study it is observed that % drug release after 6 hrs was found to be 83.13±0.47%, 87.74±0.44%, 97.82±0.39% with F1, F2, and F3 formulation respectively. In conclusion, results of pre-formulation studies showed the feasibility of Nevirapine to develop a vaginal nanoparticle dosage form which expected to reduce the systemic toxicity of the drug.
REFERENCES